POSTCOPULATORY SEXUAL SELECTION AND THE EVOLUTION OF MALE PREGNANCY IN THE GULF PIPEFISH

A Dissertation

by

KIMBERLY ANN PACZOLT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Zoology

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Approved by:

Chair of Committee,	Adam Jones
Committee Members,	Gil Rosenthal
	Bruce Riley
	Thom Dewitt
Head of Department,	Jack McMahan

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ABSTRACT

Postcopulatory Sexual Selection and the Evolution of Male Pregnancy in the Gulf Pipefish. (May 2012)

Kimberly Ann Paczolt, B.S., University of Illinois Urbana-Champaign

Chair of Advisory Committee: Dr. Adam G. Jones

Male pregnancy is a complex and energetically costly form of male parental care found exclusively in the fish family Syngnathidae, which includes pipefishes, seahorses, and seadragons. The novelty of the male brood pouch raises questions about how this trait evolved and what role it plays in sexual selection. One possibility is that brood pouch evolution was partially shaped by postcopulatory sexual selection.

We used the sexually dimorphic Gulf pipefish, *Syngnathus scovelli*, to test for postcopulatory sexual selection within broods and tradeoffs between successive male pregnancies as a function of female attractiveness. Offspring survivorship within a pregnancy was affected by the size of a male's mate, the number of eggs transferred, and the male's mating history. These results indicate the males invest more resources into broods from large, attractive mates to the detriment of future broods. Next, we investigated the effects of food limitation on male parental care strategies. Our data suggests that male Gulf pipefish sacrifice investment in future reproduction, via somatic growth, in favor of current reproduction. A positive relationship between number of failed eggs and male growth rate in our low-food treatments suggests that males may derive an energetic benefit from unsuccessful eggs in the brood pouch. Finally, we used

a paired design, to investigate the effect of perceived female attractiveness on offspring survivorship. We found that, in general, males prefer the largest female available. Within a replicate, we found that the male that preferred their mate more, regardless of stimulus female size, also had higher offspring survivorship in the resulting brood. This result is exciting because it shows not only that cryptic male choice affects offspring survivorship in Gulf pipefish but also that mate preference can have a prolonged effect on an individual's reproductive success. Ultimately, our data suggests that the evolution of the brood pouch has produced a trait that not only nurtures the offspring but also exert cryptic male choice.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Adam Jones, for his patience, good humor, and guidance over the course of my graduate degree. He has given me the room to develop as a scientist but has, on many occasions, offered suggestions that have proven critical in the development of some experiment or manuscript. He has been a champion of me and my work and has helped me succeed in ways that I would not have expected. I would also like to thank my other committee members, Dr. Thom DeWitt, Dr. Bruce Riley, and Dr. Gil Rosenthal for their confidence in me and their insightful comments about my research and advice in achieving my career goals.

I would like to thank my fellow graduate students in the Department of Biology, particularly my classmates who have shared the graduate school experience with me. In the Jones Lab, I have found a group of graduate students who are both friends and colleagues. I think it is unlikely that I might ever again work with another group of such remarkable characters.

I have also worked with a number of extraordinary undergraduates. In particular, Randall Carter, Cristin Harper, Billy Martin, and Richard Lam assisted me in the experiments described in my dissertation. Without them, I would have never been able to leave the fish care facility, so I would like to thank them for helping me have a life.

Finally, I would like to thank my family. All of my gratitude to my mother for her understanding, support, and tact, particularly when she didn't quite know what to expect from my chosen career but encouraged me anyways. I would also like to thank

my father whose enthusiasm about my research and pride in my accomplishments has been both endearing and supportive. Some might give the title “World’s Best Dad” to the male pregnant fishes I study but I have a different nominee.

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CHAPTER I

INTRODUCTION

Traditionally, the study of sexual selection has largely focused on the dynamics between male and female leading up to copulation (Darwin 1859). It wasn't until the 1970s that the scientific community began to recognize how events occurring during and after copulation could affect reproductive success and sexual selection (Parker 1970). Although we expect mechanisms of postcopulatory sexual selection to be common (Eberhard 1996), many researchers still consider only the role of the precopulatory phase of sexual selection in their study system, which may produce an incomplete picture of how sexual selection acts in that species.

Postcopulatory sexual selection is the result of any mechanism occurring after copulation begins that biases the reproductive success of one individual over another. As in precopulatory sexual selection, postcopulatory sexual selection can occur as the result of interactions within a sex, usually as sperm competition (Parker 1970), or between sexes, usually as cryptic female choice (Eberhard 1996). Sperm competition includes defensive and offensive adaptations of the genitalia, sperm, seminal fluid, and other reproductive traits that help one male's sperm fertilize more eggs than another male's sperm and has been characterized in many taxa across the animal kingdom (Birkhead and Møller 1998). In a number of studies, sperm size, number, viability, longevity, swimming duration and speed have been correlated to reproductive success (Birkhead and Møller 1998, Snook 2005), however the evolving trait in sperm

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This dissertation follows the style of *Evolution.*

competition is not always the sperm itself. Famously, accessory gland proteins found in *Drosophila* seminal fluid can stimulate egg production or ovulation in the female and ultimately reduce female longevity (Wolfner 2002, Gillott 2003). Cryptic choice may be achieved by morphological, physiological, or behavioral mechanisms that affect the number of gametes transferred, gamete usage, or embryo survivorship (Eberhard 1996). Females of some species can alter male reproductive success by altering the timing of sperm or spermatophore ejection (Pizzari 2004). In many insect species, females have specialized sperm storage organs called spermathecae which are used to sort and store sperm from different males and allow the female to control which male's sperm are used for fertilization (Eberhard, 1996, Pitnick et al 1999). In species with parental care, the caring parent may exert cryptic choice by differentially allocating resources to offspring from a preferred mate (Burley 1988, Sheldon 2000).

Previous studies in postcopulatory sexual selection have focused on species with traditional sex roles, where males compete for access to females. By studying postcopulatory sexual selection in a sex-role reversed species, we can make a significant contribution to our current understanding of postcopulatory sexual selection because in sex-role reversed species we are separating the effect of sex role, where one sex is choosy and one sex is competitive, from the differences inherent to the very definition of sex, in particular the differences in gametic investment. The use of sex-role reversed species to examine basic tenants of sexual selection theory has previously been used in regards to mate choice theory and mating system theory (Jones et al 2000, Jones and Avise 2001, Berglund and Rosenqvist 2003), this test is even more crucial in postcopulatory sexual selection where the focus is on the fate of individual gametes.

The family Syngnathidae, which includes the pipefish seahorses and seadragons, has gained scientific notoriety for the complex form of parental care, male pregnancy, found only in this group. Over the past 30 years, pipefish research has focused on understanding the evolutionary consequences of male pregnancy, particularly in regards to sexual selection. Many pipefish species are sex-role reversed, meaning that female reproduction is limited by access to males, and as a result females become the competitive sex while males become the choosy sex. Within these species, research has largely focused on three areas: mate choice behavior, genetic mating systems, and the function of the brood pouch. In many pipefish species, males prefer larger, more ornamented females (Berglund et al 1986, Berglund and Rosenqvist 1993, Berglund 1995, Partridge et al *in prep*). Not only are these females more successful in gaining mating opportunities, but they also generally produce more and heavier eggs (Berglund et al 1986, Berglund and Rosenqvist 1990, Ahnesjö 1996). The success of large female pipefish females in behavioral studies has been reflected in the characterization of the genetic mating system of natural pipefish populations using microsatellite markers. These genetic analyses have also revealed a wide array of mating systems in syngnathids, including monogamous, polyandrous, and polygynandrous species, and have provided a testing ground for metrics of the mating system, such as the Bateman gradient (reviewed in Jones and Avise 2001). Studies in a variety of pipefish species have shown that the male provides aeration, protection, and osmoregulation to his brood through the brood pouch (Azzarello 1991, Haresign and Schumway 1981, Partridge et al 2007, Quast and Howe 1980, Ripley 2009, Ripley and Foran 2009). Interestingly, the movement of macromolecules such as amino acids in both directions between the male and his offspring (Sagebakken et al 2010, Kvarnemo et al 2011) reveals the close relationship between the pregnant male and his brood and provides a potential

mechanism for the male to exert his preference through differential allocation of paternal resources.

Although pipefish have been used as a model for sexual selection, few studies have focused specifically on the role of postcopulatory sexual selection in these species, despite the fact that pipefish are, in some ways, more amenable to studies of postcopulatory sexual selection than many species with traditional sex roles. The brood pouch of many pipefish species is transparent and the large eggs are held stationary by a temporary "egg-crate" tissue produced by the male (Figure 2.1b, c, d). As a result, eggs can be counted by eye through the flaps of the brood pouch. We can take advantage of these traits to non-invasively track the fate of individual transferred gametes during the course of the pregnancy, and as a result we can quantify the transfer of gametes and survival of embryos in a way that is unachievable in most species with traditional sex roles.

Previous studies have investigated postcopulatory sexual selection in the multiply mated broad-nosed pipefish, *Syngnathus typhle*. In this species, males may mate with one or more females during a brooding cycle, producing broods that are either full or half sibling. Consequently, offspring survivorship may be affected by either egg competition or cryptic male choice in this species. Larger females transfer more eggs than small females and have more surviving offspring; however the proportion of survivorship does not differ between small and large females (Partridge et al 2009, Mobley et al 2011). Additionally, males that mate multiply have higher embryo survivorship (Sagebakken et al 2011). Although these studies begin to inform us about the factors that affect offspring survivorship in this species, it has proven difficult to effectively separate the effects of intra- and intersexual postcopulatory sexual selection

in order to explain how postcopulatory sexual selection is acting to produce the observed patterns.

The gulf pipefish, *Syngnathus scovelli*, is an even simpler and more efficient model for postcopulatory sexual selection in a pipefish. Male gulf pipefish only mate with one female per brooding period (Jones et al 1997), eliminating the possibility of direct egg competition between groups of half-siblings sharing the brood pouch. So, instead of comparing the survivorship of full-sib groups within a brood, as in *S. typhle*, we can look at how survivorship changes between consecutive broods from one *S. scovelli* male. If postcopulatory sexual selection does occur in this species, it may be mediated either by males that modulate investment in the brood, differences in inherent female quality, or by females that manipulate the male's parental investment.

In these studies we address the following questions in order to better understand the evolution of the brood pouch and the nature of the interaction between the male and his brood in the singly-mated Gulf pipefish. First, does postcopulatory sexual selection occur in the Gulf pipefish? Second, how do environmental factors affect patterns of postcopulatory sexual selection in this species? Third, is postcopulatory sexual selection mediated by cryptic male choice?

CHAPTER II

POSTCOPULATORY SEXUAL SELECTION AND SEXUAL CONFLICT IN THE EVOLUTION OF MALE PREGNANCY*

Introduction

Male pregnancy is an extremely rare phenomenon, unique to seahorses and their relatives, and its existence provides unparalleled opportunities to study parent-offspring interactions and mechanisms of sexual selection from a novel perspective. In many syngnathid species, the male possesses an elaborate brood pouch into which a female deposits eggs during mating (Herald 1959, Dawson 1985). Far from being a passive bag for offspring (Figure 2-1), the male's pouch provides aeration, protection, osmoregulation, and nutrition to the developing offspring during a pregnancy that can last several weeks (Berglund et al 1986, Ripley and Foran 2006, Partridge et al 2007, Ripley 2009, Ripley and Foran 2009). The male's brood pouch is a structure of considerable evolutionary interest, because it is a complex, novel trait with no known homologs outside of the Syngnathidae (Harlin-Cognato et al, 2006), and it has substantially affected the course of evolution in syngnathid taxa (Wilson et al 2003, Harlin-Cognato et al 2006). The most consequential evolutionary impact of male pregnancy has been on the direction of sexual selection, as some syngnathids are characterized by stronger sexual selection on females compared to males, a phenomenon

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known as sex-role reversal (Berglund et al 1986, Vincent et al 1992, Jones et al 2000, Berglund and Rosenqvist 2003).

The brood pouch is traditionally seen as a structure whose main role is to nurture offspring. Recent work has established that nutrients move both from father to offspring (Berglund et al 1986, Ripley and Foran 2006, Ripley and Foran 2009) and from offspring to father (Sagebakken et al 2010) during the pregnancy, raising the hitherto unexplored possibility that the brood pouch may play a role in modulating postcopulatory sexual selection and sexual conflict (Trivers 1972, Parker et al 2002, Arnqvist and Rowe 2005). On the one hand, males could withhold or steal resources from broods with unattractive mothers to save resources for future pregnancies, resulting in tradeoffs between broods and postcopulatory sexual selection within pregnancies (Burley 1988, Sheldon 2000). On the other hand, sexual conflict could be driven by female interests if the females can transfer substances along with the eggs that stimulate the males to invest more resources in the current pregnancy (Wolfner 2002) or if certain mothers have embryos that are better able to harvest resources from the males compared to other embryos (Zeh and Zeh 2000). Whether sexual conflict is driven by male- or female-mediated effects, the demonstration of a role for the brood pouch in such processes would precipitate a major shift in thought regarding adaptive mechanisms responsible for the evolution of male pregnancy and parent-offspring interactions in this fascinating group of fishes.

We focus here on the Gulf pipefish, *S. scovelli*, which exhibits substantial sexual dimorphism and has the highest documented opportunity for sexual selection on females of any taxon (Jones et al 2001). In nature, males normally mate with a single female per pregnancy (Jones and Avise 1997, Jones et al 2001) whereas the most successful females

can mate with several males, resulting in a limited supply of receptive males. Previous studies of postcopulatory processes in a related pipefish (*S. typhle*) addressed phenomena occurring within individual pregnancies to which multiple mothers contributed (Ahnesjö 1996, Partridge et al 2009). The results showed that eggs from larger females, who produce larger than average eggs, tended to result in offspring with higher survivorship than those from smaller females (Ahnesjö 1996, Partridge et al 2009). However, these studies did not identify any male effects on offspring survivorship within the brood pouch nor did they address between-pregnancy effects in any way. Gulf pipefish provide an excellent model in which to focus on between-brood effects, while eliminating any complications from multiple mating within broods, in a strongly sexually selected species. Previous work has established that precopulatory sexual selection is important in Gulf pipefish (Jones et al 2001), so here we address the postcopulatory side of sexual selection by examining offspring survivorship within male pregnancies in light of female attractiveness and male mating history.

To ascertain the extent to which inter-brood tradeoffs play a role in Gulf pipefish reproduction, we examined brood reduction in light of a male's mating history. If sexual conflict occurs, then we expect to see tradeoffs in subsequent broods. For example, if males use brood reduction as an adaptive reproductive strategy, then we should expect males to reduce broods originating from smaller than average females to save resources for future broods originating from larger females. Similar predictions apply if sexual conflict is driven by female-mediated strategies, as some females would be expected to manipulate the reproductive investment of males at the expense of his future mates. An absence of between-brood tradeoffs would suggest a lack of sexual conflict via differential allocation of resources.

Methods

Gulf pipefish were collected near Port Aransas, Texas, USA on three occasions between June and August 2007, transported to the Texas A&M University campus in College Station, and housed in a flow-through system until they were used in the experiment. Collection date had no significant effect on brood reduction, brood size, or time spent in the lab (Tukey-Kramer HSD test, $\alpha \leq 0.05$). Most males were pregnant at the time of collection, so we housed them in groups until they gave birth. Males usually were added into the mating experiment less than 24 hours after releasing their field-conceived brood. Females were housed in female-specific tanks until they were used in the mating experiment.

Males were housed in 9.5 L aquaria, equipped with sponge filters and artificial seagrass, and fed *Artemia* nauplii twice daily. Each male was maintained in the same tank for the duration of the trial, and tanks were thoroughly cleaned between trials. Males were randomly assigned to mate with either a large (108 to 122 mm) or small (93 to 106 mm) female. The reproductive status of females was assessed by the presence of the female secondary sexual ornament, which appears as silvery-blue lateral bars on the trunk.

Each male was presented with a randomly assigned female of the appropriate size class immediately upon addition to the experiment. Male brood pouches were visually inspected daily. Of the 48 males that started the experiment, 34 accepted eggs from their first mate. The remaining 14 males were excluded based on the following criteria: death of either individual in the pair, mating outside the intended pair, or failure to mate before the experiment end date (November 4, 2007). Two more males were excluded because

they died before the brood emerged. Broods were monitored during development (see below). Males and females were maintained together until the male was moved into a brooding chamber 11 days after mating. On the day the fry emerged the female was

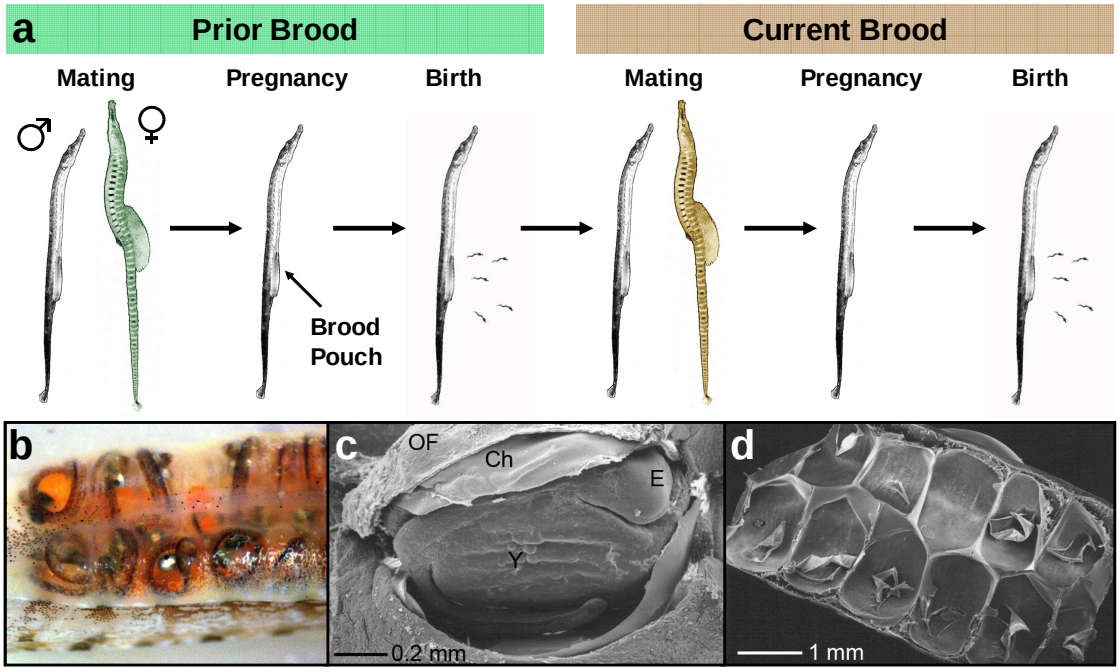

Figure 2.1. Experimental design. **a**, Our experimental design involved mating each focal male with two females in succession. We allowed the male (black image on the left) to mate with the first female (larger green individual), photographed the contents of his pouch shortly after mating and several times during the pregnancy, and permitted him to give birth. After the birth of his prior brood, we paired the male with a new female (which sometimes differed in size from his prior mate), allowed him to mate, and photographed his brood several times throughout the pregnancy. The brood pouch is an external structure on the ventral surface of the male's tail, posterior to the vent. **b**, The transparency of the brood pouch facilitated this experiment by permitting us to observe the status of each egg as the pregnancy progressed. This image shows a ventral view of a section of a male's brood pouch during the middle stages of pregnancy and is oriented in such a way that the male's head would be to the left and his tail to the right. **c**, The close connection between embryo and father facilitates a transfer of nutrients between the male and offspring in both directions. In **c**, the outer flap of the brood pouch has been removed, showing the embryo nestled within an individual chamber in the pouch. This SEM image shows the outer flap (OF), the chorion (Ch), the embryonic tissue (E), and the yolk. The chorion breaks down during the pregnancy, and the embryo is contained within a chamber of paternal tissue. **d**, A honeycomb of paternal tissue is present in the brood pouch when the embryos are removed at about the half-way point of a pregnancy. Pipefish and SEM images were provided by N. Ratterman and C. Partridge.

sacrificed by an overdose of tricaine methane sulfonate (MS-222), photographed, and preserved in formalin solution. Measurements of female total length, snout-vent length, weight, standard depth (Scobell et al 2009), maximum depth, and dorsal fin area were recorded. Up to five eggs were removed from each female, photographed, and measured for diameter.

The 30 males still in the experiment after the first pregnancy were each presented with a second mate within 48 hours of giving birth. Males were again randomly assigned to either a large or small female, and the pairs were monitored as previously described. No females were re-used during the experiment, so the second mates were independent of the first mates. Twenty-three of the 30 males accepted eggs from the second mate, and 22 males survived to maturity of the brood. The remaining seven males that did not mate were excluded from the experiment. On the day of parturition of the second brood, both the male and female were sacrificed in tricaine methane sulfonate (MS-222), photographed and preserved in formalin. Females were measured as previously described. Measurements of male total length, snout-vent length, and standard depth also were recorded at this time. At the end of the experiment, male total length ranged from 87 to 105 mm. Male and female total length were not correlated in this experiment ($N = 22$; $r^2 = 0.13$, $p = 0.107$).

On days one, seven, and 11 after mating, embryos were counted and the progress of development was assessed. The male was transferred to a small amount of water and examined under a dissecting microscope. Embryos are visible through the brood pouch epithelium, and can be counted by eye. All broods were counted at least twice to verify accuracy. Unusual looking eggs were noted, and eggs that showed no obvious development (compared to other embryos in the brood) counted towards brood

reduction. Undeveloped eggs often shrank and became more transparent during the brooding period. A time series of brood development was produced by photographing the broods on these days using a digital camera (AxioCam MRC5) attached to a stereo microscope (Zeiss Stemi 2000-C).

Embryos failing to develop can be distinguished from viable embryos by their diminished size and colour at day seven of the pregnancy (which typically lasts 12-14 days), so we measure brood reduction by dividing the number of inviable embryos by the number of eggs initially received. Similarly, we refer to offspring survivorship as the number of viable offspring divided by the number of eggs initially received, while keeping in mind that some of the eggs that failed to yield viable progeny may not have been fertilized. The photographic time series of the brood was consulted if a question arose as to the fate of a particular egg.

We controlled the size of the females with which males mated to ensure that males sometimes mated with females that differed from one another in size and sometimes mated with females similar in size to one another. Most of the analyses presented here are based on the second brood for each male (N = 22), which we refer to as the "current brood" (Figure 2.1). The purpose of the first brood (which we call the "prior brood") was to establish a known mating history for each male. We first addressed whether or not males exert precopulatory mate choice; second, we quantified postcopulatory sexual selection by examining relationships between offspring survivorship and female size; third, we examined factors affecting offspring survivorship within broods, with a particular emphasis on tradeoffs between broods; and finally, we used an individual-based model to investigate the circumstances under which a brood reduction strategy would be adaptive in nature.

Because of the large number of variables measured in this study, we used stepwise regression as an exploratory tool to evaluate which variables should be included in a path analysis. The response variable was current brood reduction, and all included variables had individual *p*-values less than 0.05 in the stepwise regression analysis. Based on these results, we kept variables describing adult total length, reluctance to mate, brood size, and prior brood reduction. All variables were transformed to meet a normal distribution before statistical analysis. Univariate and stepwise regressions were performed in JMP 7.

In our path analysis, all variables retained from the stepwise regression analysis were connected to our focal variable, current offspring survivorship. Other connections were included to represent all likely biological interactions between the measured variables. We estimated partial regression coefficients, standardized to one standard deviation, by using the maximum likelihood procedure implemented in the computer program AMOS 5.0.1. The path analysis model resulted in a chi-squared value of 13.05, leading to a P of 0.522, which implies that the hypothesis of a perfect fit of the model cannot be rejected. This model explained 84% of the variance in current offspring survivorship. The model fit achieved an RMSEA value of < 0.001, below the 0.05 value expected for a model with excellent fit (Steiger and Lind, 1980). Overall, the measures of fit for the path analysis suggest that the estimated model is an accurate representation of our data.

Results

With respect to precopulatory choice, our results indicate that males prefer to mate with larger females. Each mating trial was essentially a no-choice preference

experiment, and we found that males' reluctance to mate was significantly shorter for larger females (Figure 2.2a), a result that was even more pronounced when we

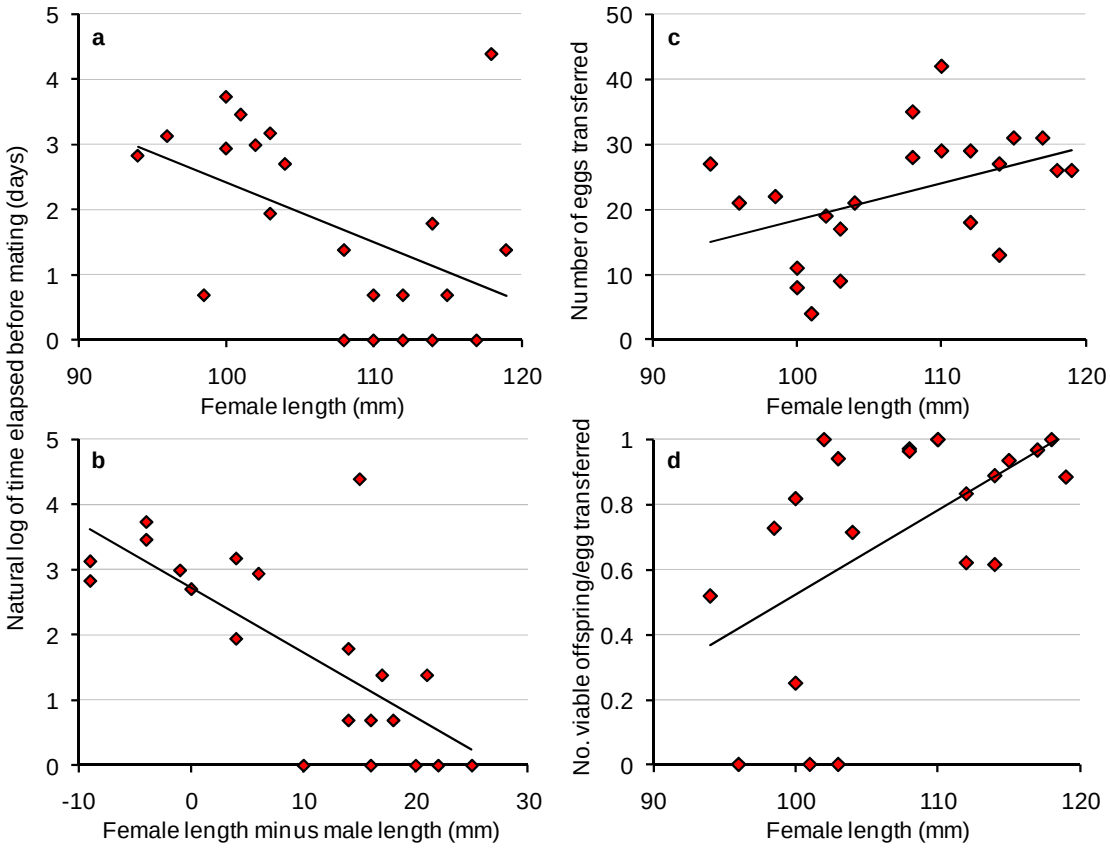

Figure 2.2. Evidence for precopulatory mate choice and postcopulatory sexual selection by males of the Gulf pipefish. **a**, We observed a strong negative correlation between a female's total length and the time that elapsed before mating took place for male-female pairs (N = 22, r^2 = 0.23, p = 0.025). **b**, The pattern was more pronounced when we took into account the female's length relative to the male's length (N = 22, r^2 = 0.53, p < 0.001), suggesting that males assess female size relative to their own body size. **c**, Sexual selection arising from the number of gametes transferred is normally considered a form of postcopulatory sexual selection, even though technically it occurs during copulation. We observed a significantly positive relationship between female total length and number of eggs transferred to the male's pouch (N = 22, r^2 = 0.20, p = 0.038). **d**, Males also experienced higher levels of offspring survivorship when they mated with larger as opposed to smaller females (N = 22, r^2 = 0.56, p = 0.007). Thus, larger females, which are preferred by males as mates, also transfer more eggs per mating, and the embryos from larger mothers experience enhanced survivorship within the male's pouch.

considered female size relative to male size (Figure 2.2b). Thus, precopulatory mate choice favours larger females, and the pattern is very strong, as might be expected for a highly sexually dimorphic species like the Gulf pipefish (Jones et al 2001). This observation is consistent with field-based parentage studies, which show that the largest, most ornamented females enjoy the highest share of maternity (Jones et al 2001).

Our second goal was to investigate the nature of postcopulatory sexual selection in Gulf pipefish. Our results showed that pregnancies exhibited substantial variation with respect to the number of eggs transferred per copulation (range = 4-42; mean = 22.5; variance = 89.8) and survivorship of eggs in the brood pouch (range = 0-1; mean = 0.71; variance = 0.12), setting the stage for postcopulatory sexual selection. The level of brood reduction in Gulf pipefish (i.e., 0.29 on average) is very similar to the approximately 30 percent reduction that has been observed in *S. typhle* (Ahnesjö 1996, Partridge et al 2009), the only other pipefish species for which data are available. If postcopulatory sexual selection occurs in pipefish, then one very important question is whether it reinforces or opposes precopulatory sexual selection (Andersson and Simmons 2006, Hunt et al 2009). Other studies of postcopulatory sexual selection in a wide range of taxa have provided mixed results on this issue (Andersson and Simmons 2006). Our results show that males prefer to mate with larger females (who also tend to have more pronounced secondary sexual characters) and that postcopulatory sexual selection acts in the same direction. We observed a strong positive correlation between number of eggs transferred and female size (Figure 2.2c). We saw the same pattern for offspring survivorship (Figure 2.2d). In short, larger females transferred more eggs per mating, and the resulting embryos experienced a greater probability of surviving to

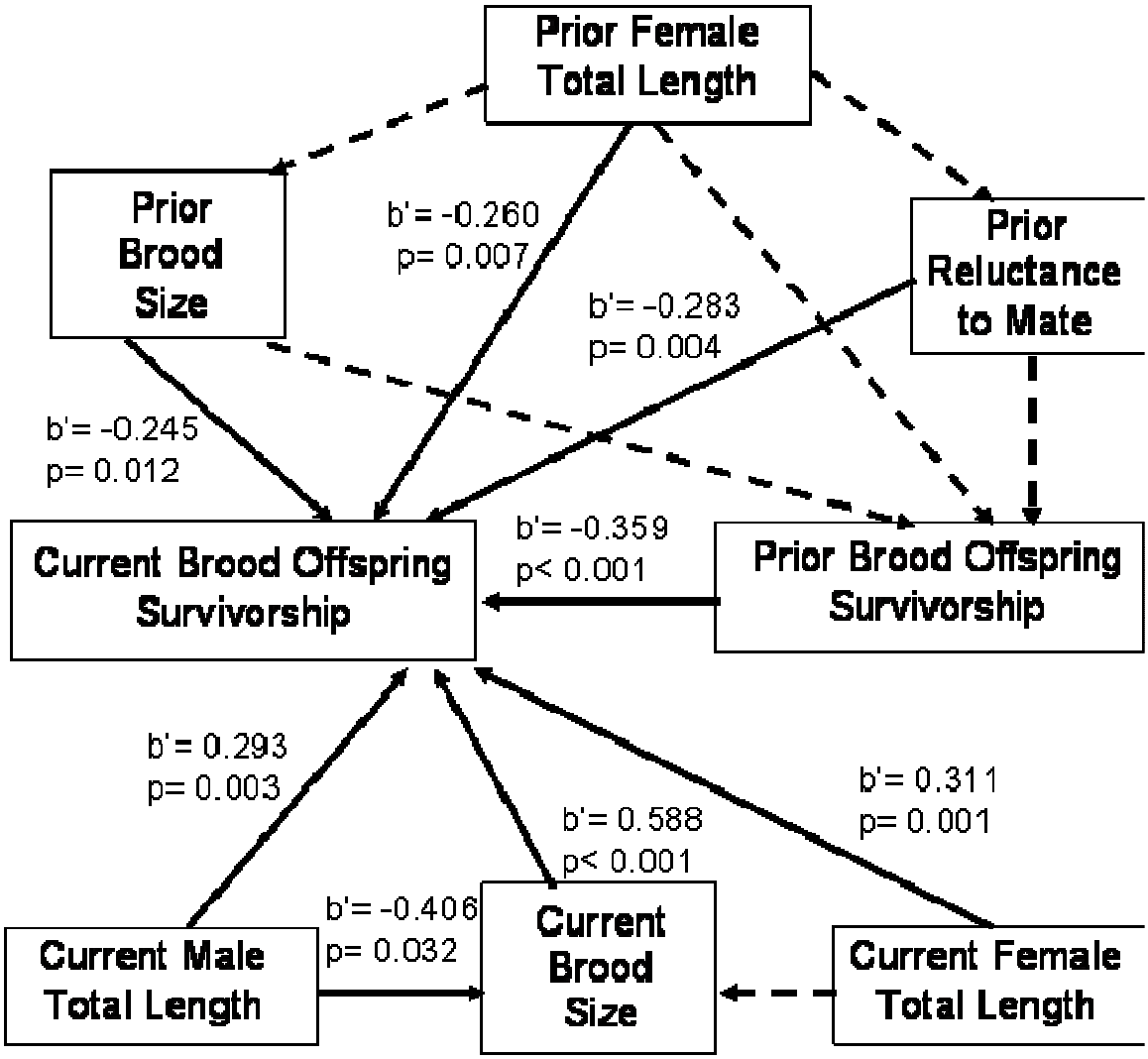

Figure 2.3. Path analysis diagram. Solid arrows represent significant partial regressions, whereas dashed arrows show relationships that were included in the analysis but did not achieve statistical significance. For each significant relationship, we show the partial regression coefficient (b') and the *p*-value near the appropriate solid arrow. The key result illustrated in this figure is that the survival of embryos in the current brood depends on features of the previous brood. Most importantly, current offspring survivorship was negatively correlated with prior offspring survivorship and negatively correlated with prior female length. Thus, if a male mated with a large, attractive female during his first brood and experienced high offspring survivorship during that brood, then he typically experienced low survivorship in his next brood. This result shows that inter-brood tradeoffs occur and that they are consistent with expectations from an adaptive male strategy of cryptic choice. The prior brood served its purpose to establish known mating histories for the males, giving us much better power to detect significant effects in the current brood.

parturition. Thus, postcopulatory sexual selection reinforces precopulatory sexual selection in Gulf pipefish.

Our most important result with respect to postcopulatory sexual selection and sexual conflict is that current offspring survivorship is dependent on characteristics of the prior brood (Figure 2.3). In particular, current offspring survivorship is negatively correlated with prior female size and prior brood size, suggesting that valuable (and energetically expensive) previous broods hinder a male's ability to invest in current broods (Figure 2.3). Similarly, current offspring survivorship is negatively correlated with prior offspring survivorship (Figure 2.3), suggesting that males are capable of reducing investment in some broods to save resources for future reproduction and that investment in a valuable brood decreases survivorship in subsequent broods. The path analysis also provides additional statistical confirmation that characteristics of the current pregnancy affect brood reduction. For example, female size and brood size in the current brood positively affect current offspring survivorship (Figure 2.3), as noted above.

Discussion

What do these results imply regarding the function of the brood pouch during male pregnancy? Importantly, we can decisively rule out the view that the brood pouch is simply a passive receptacle in which embryos develop without any important input from the male. Under this view, the reduced survivorship of eggs originating from small females could be explained if small females produce eggs with exceptionally low viability. However, several observations suggest that this explanation is inadequate.

First, a male's mating history influences offspring survivorship, a pattern that would be impossible without interactions between the male and offspring, mediated by the placenta-like connection provided by the brood pouch (Vincent et al 1992). Second, we intentionally avoided using extremely small females in our experiment, so all but one of our "small" females was above the average female size observed in our field population at the onset of the breeding season (female field data from 12 June 2007: $N = 54$; mean length = 96.5mm, S.D. = 9.89, range 80-120mm; experimental females for current brood: $N = 43$, mean length = 107.3mm, S.D. = 7.6, range = 93-119mm). The average survivorship of the eggs originating from the smaller females (94-104mm total length) in our experiment was only 49.7 percent, while 89.0 percent of the eggs from larger females survived. It seems unlikely that the females we used, which had probably been reproductively active in the field for quite some time before collection, would have such low intrinsic egg viability. Finally, we found a weak negative correlation between offspring survivorship and the length of offspring at birth ($N = 20$, $r^2 = 0.23$, $p = 0.03$), suggesting that the surviving eggs from females whose broods were reduced resulted in offspring of at least average quality. Hence, the eggs from small females appear not to be of low enough quality to preclude the production of quality offspring. This latter result brings up the possibility that sibling competition could be occurring in the brood pouch. Our results suggest that sibling competition may be occurring at some level, because offspring from broods with high survivorship were slightly smaller at birth than offspring from broods with lower survivorship. However, in a multiple regression of offspring length on brood size, offspring survivorship, and female egg diameter, only offspring survivorship showed a statistically significant association with offspring length ($N = 20$; egg diameter: $p = 0.68$; brood size: $p = 0.43$; offspring survivorship: $p = 0.001$). In addition, a sibling competition model is directly at odds with our observation that

larger broods experienced greater offspring survivorship, so sibling competition alone cannot explain our most important results.

The simplest explanation for our results is that broods with larger mothers are energetically more costly for males. These costs could be manifested in two ways: either males are selected to invest more resources in embryos from large, attractive mothers as an adaptive strategy of cryptic male choice or larger females (or their offspring) have evolved a mechanism that induces greater reproductive investment by males. Overall, the cryptic male choice hypothesis is most consistent with our data, because all of our observations can be explained by discrimination against smaller females at every phase of pre- and postcopulatory sexual selection. Moreover, the mechanism would be relatively simple, as males could reduce the rate at which resources are transferred from the male to the broods from less attractive females, increasing competition among siblings and reducing offspring survivorship. This conclusion is further bolstered by recent observations indicating that pipefish males actually are capable of harvesting nutrients from their broods. In *S. typhle*, an experiment involving the incorporation of ^{14}C-labelled amino acids into the eggs of females before mating showed that after mating some of the nutrients from those eggs pass from the embryos through the male's brood pouch to be incorporated into his liver and muscle tissue (Sagebakken et al 2010). Thus, male pipefish possess a mechanism to take resources from their broods, and the male-mediated strategy of brood reduction that our data illustrate is an example of sexual conflict, analogous to infanticide or filial cannibalism (Arnqvist and Rowe 2005). Males are partially, or in some cases completely, aborting the offspring from smaller females, clearly at the expense of the reproductive interests (and Darwinian fitness) of those females. The strategy appears to be beneficial to the males, since their future broods,

which should have larger mothers on average, experience enhanced offspring survivorship. This clash of reproductive interests of the sexes is the very crux of sexual conflict (Arnqvist and Rowe 2005), so our results raise the hypothesis that females should be selected to somehow resist this male strategy.

The other possible explanation for our results, which seems less likely but would be equally interesting, is that inter-brood tradeoffs are determined mainly by some sort of conflict driven by female reproductive strategies (Parker et al 2002). Perhaps larger females produce offspring that are more capable of harvesting resources from males or transfer proteins along with the eggs that increase a male's investment in offspring at the expense of future broods, in a manner analogous to *Drosophila* accessory gland proteins (Wolfner 2002). Under this scenario, males would see some of the same tradeoffs that we observed here. However, this model cannot completely explain our data, because while a female-driven reproductive strategy would predict that a brood from a small female would do poorly on the heels of a brood from a large female, the universally poor performance of broods from small females is most easily explained by a male-mediated strategy (Figure 2.2d). In addition, if females are driving the pattern, then we would expect the effects to be most severe immediately after a pregnancy with a large mother, diminishing over time in unmated males as they regained resources. However, we found the opposite pattern. Among males with large prior mates, a male's reluctance to mate was negatively correlated with offspring survivorship in the current brood ($N = 11$; $r^2 = 0.53$; $p = 0.011$). This pattern is easily explained under the cryptic choice hypothesis if the males selectively reduced the broods from less attractive females (with whom they are also reluctant to mate). Thus, even though female-mediated effects probably play some role, brood reduction clearly is dependent on a male's mating history, and the

pattern we observed is most consistent with the hypothesis that pipefish reproduction includes a substantial component of cryptic male choice through differential abortion or allocation of resources.

If males are capable of manipulating female broods, as our data suggest, then a key question is whether or not such a strategy is likely to be adaptive in natural populations. Intuitively, the best strategy for males would be to mate with large, attractive females every pregnancy. However, several lines of evidence suggest that this strategy may be impossible. For example, the average potential reproductive rates of females are only about twice those of males (Scobell et al 2009), so the largest females have the potential to become egg depleted. Assuming that the preferred females are above average in phenotype, there may be an insufficient number of preferred females to fill the available empty brood pouches at any given time. For example, if preferred females are one standard deviation above the mean, only about 16 percent of females would fall into this category. Perhaps the threshold is lower, but it would have to be close to the mean for the females to have sufficient eggs to keep all males pregnant in a population with an equal sex ratio. The situation is compounded by the observation that pipefish sex ratios fluctuate dramatically, often showing a large excess of males. In a 15-month study of pipefish demographics (Brown 1972), five months showed significant departures from equal sex ratios and in four of the five cases males outnumbered females. The most severe shortages of females (0.47 and 0.51 females per male) occurred in June and July, during the peak of Gulf pipefish reproductive activity, and the proportion of pregnant males also decreased during these months, suggesting that males were having difficulty finding attractive females (Brown 1972). Overall, it appears very likely that males will

sometimes be unable to find preferred females ready to mate, but a large number of non-preferred females will virtually always be available.

The results of this study are significant at two levels. First, postcopulatory sexual selection has been virtually unexamined in sex-role-reversed taxa. If cryptic choice is a general mechanism of sexual selection, then we should expect it to evolve in sex-role-reversed species as well as the more commonly studied species with conventional sex roles. Our results show that such a mechanism has evolved in Gulf pipefish, despite the fact that this species has effective mate choice before mating, implying that cryptic choice really is a process of fundamental significance. Second, our results bear on the adaptive significance of the brood pouch. At face value, the pouch appears to be a structure that evolved to nurture and provide for offspring, and indeed it does serve this function. However, it also may grant the male better control over reproduction. Males seem to be able to adaptively affect their investment in broods as a function of the value of the pregnancy. Perhaps they simply invest fewer resources in broods originating from smaller females (resulting in competition among siblings within the pouch for resources), but males also have the ability to exploit the reproductive contributions of some females by actively absorbing embryos from less valuable broods (Sagebakken et al 2010). Thus, the brood pouch serves a more complicated role in pipefish reproduction than previously believed, certainly providing parental care but also fighting on the front lines in a conflict between the sexes.

CHAPTER III

THE EFFECTS OF FOOD AVAILABILITY ON GROWTH AND OFFSPRING SURVIVORSHIP IN PREGNANT MALE PIPEFISH

Introduction

Male pregnancy is a unique form of male parental care exclusively found among the pipefishes, seahorses, and seadragons of the Family Syngnathidae. In these species, mating involves the transfer of eggs to the male's brood pouch, a specialized epithelial structure on the ventral surface, after which the male cares for the developing embryos until they emerge as independent juveniles. Male pregnancy is a complex and energetically costly physiological function that provides protection, aeration, osmoregulation, and nutrition for the male's developing brood (Azzarello 1991, Haresign and Schumway 1981, Partridge et al 2007, Quast and Howe 1980, Ripley 2009, Ripley and Foran 2009). Paternal investment during the pregnancy can be differentially allocated based on the attractiveness of the females, resulting in tradeoffs between current and future reproduction (Paczolt and Jones 2010). The magnitude and consequences of such tradeoffs probably vary dramatically depending upon the ecological and social environment in which the pregnant male finds himself. Hence, to better understand the importance of postcopulatory brood survivorship as an evolutionary factor in pipefish, we would like to obtain a greater appreciation of how ecological factors affect the outcome of male pregnancy.

Thus far, very little empirical effort has been directed toward resolving how patterns of differential allocation in pregnant male syngnathid fishes vary based on

fluctuations in ecological and population level factors and ultimately how such relationships may affect sexual selection. The goal of the present study is to investigate the effects of one such environmental variable, food availability, on the tradeoff between somatic growth and the survivorship of offspring from large, attractive females and small, unattractive females. Tradeoffs between investment in reproduction and investment in growth or condition are effectively tradeoffs between current and future reproduction because larger, healthier, or heavier individuals typically produce more offspring (Carlisle 1982, Roff 1992, Stearns 1992), a relationship previously observed in pipefish (Berglund and Rosenqvist 1990, Ahnesjö 1992, Paczolt and Jones 2010).

Tradeoffs between investment in parental care and growth or condition are common across taxa with parental care. In some cases, females sacrifice investment in condition in favor of reproduction. In northern grass lizards, *Takydromus septentrionalis*, females that produce four or more clutches in a season gained less weight than females producing fewer clutches, even though investment in weight gain would increase fecundity the following year (Ji et al 2007). In other cases, females sacrifice reproduction in favor of condition, as in female mountain goats (Hamel et al 2010) or red-backed salamanders (Yurewicz and Wilbur 2004). The caring parent may even end up in better condition than their non-caring counterparts. In the assassin bug, nest guarding males were heavier than non-guarding males on the day of hatching, despite the fact that nest guarding males were never observed leaving the eggs to capture prey (Thomas and Manica 2003). On the other hand, some environments are so resource limited that both female condition and reproduction are impacted. In one such example, white tailed-deer in a low resource environment reduced both female weight and fawn survivorship (Therrien et al 2007). In species with biparental care, the tradeoff between

investment in self and reproduction may fall entirely to one of the caring parents. For instance, manipulation of brood size and paternal effort impacted the condition of female blue-footed boobies but had no effect on male condition (Velando and Alonso-Alvarez 2003).

The dynamics of differential allocation in male pregnancy may be conceptually most similar to the filial cannibalism often found in nest building fishes (Manica 2003). Both cases are characterized by taxa with indeterminant growth, exclusive male parental care, and a mechanism by which the male can recover some of the resources invested in the offspring, either by consuming eggs, as in filial cannibalism (Sargent 1992), or by absorbing nutrient from the eggs through the brood pouch, as has been shown in the broad-nosed pipefish (Sagebakken et al 2010). A link between filial cannibalism and paternal condition has been observed in many paternally caring species, such as the sand goby (Lindström 1998), fantail darter (Lindström and Sargent 1996), bluegill sunfish (Neff 2003), freshwater goby (Okuda et al 2004), and threespine stickleback (Mehlis et al 2009). Other species have failed to show such a pattern, however (Manica 2003). Increased filial cannibalism has also been observed in males of smaller size classes (Lindstrom 1998, Klug 2009), possibly indicating that the benefits of filial cannibalism to future reproduction is greater for smaller compared to larger individuals.

Here we utilize the Gulf pipefish (*Syngnathus scovelli*), to investigate the effects of environmental variables on male tactics of parental care investment. In the Gulf pipefish, males mate with one female per brooding period, while females can mate with several males (Jones and Avise 1997; Jones et al. 2001; Scobell et al 2009). Once pregnant, the male carries the brood for approximately fourteen days. Survivorship of

the eggs is biased in favor of large females, and the difference between successful and unsuccessful embryos is clear midway through the pregnancy (Paczolt and Jones 2010).

In this paper, we use a two-factor experiment to examine the effects of mate size and food availability on male growth rate and offspring survivorship during pregnancy in Gulf pipefish to address three major questions. First, how do patterns of offspring survivorship change with resource availability? Second, do males exhibit tradeoffs between somatic growth and reproduction? And third, is embryo size affected by the amount of resources available to the pregnant male?

Methods

Pregnant males and sexually mature females were collected by seine from shallow waters in the Gulf of Mexico near Aransas Pass, Texas in June and July, 2010. Fish were transported back to our live animal facility at Texas A&M University in College Station, Texas. Sexual maturity in females was evaluated by the presence of silvery-blue lateral bands, a secondary sexual trait that occurs only in mature females. Males and females were group housed in a flow-through, saltwater system until they were used in the experiment. Pregnant males were held until they gave birth, so all males used in this experiment were not pregnant at the beginning of the experiment but had been pregnant previously, ensuring that they were reproductively active.

Experimental males were randomly assigned to one of two feeding regimes (low or high) and moved into individual 9.5 liter tanks equipped with artificial seagrass and a sponge filter. Males on the high food treatment (n = 45) were fed four times a day with two day old enriched *Artemia* nauplii and supplemented with copepods once per day.

Males on the low food treatment (n = 45) were fed twice a day with two day old enriched *Artemia* nauplii. Males were maintained on this feeding regime in isolation for seven days, at which time they were randomly assigned to a mate treatment. Our food treatments are relative: high food males had access to twice as much food per day as low food males and were fed more frequently. The levels of food were chosen so that high food males would always have a surplus of food while low food males would usually clear the available food between feedings.

Males were assigned to one of three mate treatments: large female mate (n = 19, female total length 110-128 mm), small female mate (n = 38, female total length 86-103 mm), or no mate (n = 32). To keep females in good condition, each female was housed individually in a 9.5 liter tank, under a high food regime, during most of the day, except during her mating interactions with the male. Female size did not differ between food treatments at any time point (Student's t-test, trial day 1, n = 32, $P = 0.7312$; pregnancy day 1, n = 32, $P = 0.6958$). For the mating encounters, a single female was moved into each male's tank approximately 45 minutes after the last feeding of the day, allowed to remain with the male throughout the night, and transferred back to her own tank immediately prior to the first feeding of the next day. In the lab, most courtship activity occurs shortly after lights on, so this experimental design provides ample opportunity for pairs to mate. Mating pairs took between 1 and 21 days to mate with a mean of 7.18 days. Latency to mate did not differ between the large and small mate treatments or the high and low food treatments (two-way ANOVA**,** mate: $F_{1,32} = 0.90$, $P = 0.35$; food: $F_{1,32} = 0.28$, $P = 0.60$; mate*food: $F_{1,32} = 31.64$, $P = 0.07$). Male-female pairs had visual contact while separated, but were visually and chemically isolated from all other pairs. Pairs were maintained in this stage of the experiment for up to 28 days. Fifteen

replicates were excluded because the pair did not mate within the time allowed. Replicates were no more likely to fail in the low food treatment than in the high food treatment (Fisher's exact test, $P = 0.54$). This pattern also cannot be explained by failure of these female to produce eggs during the trial, because nine of the females in the failed replicates had large, apparently mature eggs in their ovaries at the time they were dissected. Thirteen of these pairs were in the small female mate treatment, which may indicate that these males refused to mate with unattractive females.

Male reproductive state was assessed daily, and females were removed and sacrificed the day after the male became pregnant. Pregnant males were maintained on their assigned food treatments for an additional eight days, at which point the male was also sacrificed. Offspring survivorship was then assayed by dissecting the brood pouch and counting the number of normal and failed eggs. Our previous results showed that failed eggs stay in the brood pouch throughout the pregnancy, and that these eggs can be easily distinguished from normal eggs midway through pregnancy (Paczolt and Jones, 2010). Five embryos were randomly chosen from the brood and digitally photographed. Embryo length and yolk area were measured using ImageJ (Abramoff 2004). Five mature eggs were also dissected from the female's ovary, photographed, and measured for area using ImageJ.

To assess the success of each brood, we calculated residual offspring survivorship from a linear regression of number of surviving offspring on total number of eggs initially received by each male. Number of surviving offspring is strongly correlated with number of eggs initially received (linear regression, $R^2 = 0.919124$, $F_{1,32} = 353.6664$, $P < 0.0001$), so residual offspring survivorship provides a measure indicating the extent to which a male had more offspring than expected survive given his

initial brood size. Positive values indicate higher than expected offspring survivorship, given the initial number of eggs, whereas negative values indicate lower survivorship than expected. One replicate was an extreme outlier in this transformation and was excluded from further analysis.

Males and females were measured on the first day the female was added to the experiment and on the day after the pair mated. The males also were measured on the day they were added to the experiment and on the ninth day of pregnancy, which was the day the male was sacrificed. Measurements of control males mimicked those of experimental males: the "no mate" males were measured after one week on the food treatment, which coincides with the day the female was added in experimental treatments, and again nine days later, which would coincide with the day a pregnant male was sacrificed. Total length and standard depth (taken directly anterior to the dorsal fin) were measured in both sexes. The fish were lightly anesthetized using clove oil prior to each measurement. Fish were sacrificed with an overdose of MS-222. Since depth is highly correlated with total length in both sexes (linear regression, male: $R^2 = 0.581$, $F_{1,64} = 88.76$, $P < 0.0001$; female: $R^2 = 0.314$, $F_{1,32} = 14.668$, $P = 0.0006$), residual depth measurements were used for all statistical analyses.

Growth rate was calculated as the change in total length per day for the periods of time during and directly before pregnancy. Standardized total length growth rate was corrected for the asymptotic growth anticipated in fish with indeterminate growth. As expected, in the no mate treatment, larger males grew less than smaller males (linear regression, $R^2 = 0.318$, $F_{1,30} = 14.0127$, $P = 0.0008$); however, this pattern was not statistically significant in pregnant males (linear regression, $R^2 = 0.0748$, $F_{1,32} = 2.5878$, $P = 0.1175$). To correct for the effect of size on growth rate, we standardized growth

rate by taking the residual of the growth rate in pregnant males from the regression line fitted to growth rate in the control (no mate) males. For this paper, we will use the term "pregnant growth rate" to apply to male standardized total length growth rate during pregnancy and "non-pregnant growth rate" to refer to male standardized total length growth rate before pregnancy.

Statistical analyses were performed in JMP, Version 9.0 (SAS Institute, Inc.). Eight replicates were excluded from analyses because either the male or the female died prematurely. In total, our analyses include 7 individuals in the large mate, high food treatment, 8 individuals in the large mate, low food treatment, 9 individuals in the small mate, high food treatment, and 10 individuals in the small mate, low food treatment. An additional 16 males were included in each of the control treatments (no mate, high food and no mate, low food).

Least squares linear models were constructed to describe the factors affecting our variables of interest. We first used stepwise regression analysis to choose the variables included in the model (Sokal and Rohlf 1995). The stepwise regression included all appropriate effect variables such as food and mate treatment, male and female total length, brood size, growth rate, and offspring survivorship. Variables that had significant effects on our variable of interest in the stepwise regression were incorporated into the least squares linear regression model along with all possible interaction terms. Nonsignificant interaction terms were then removed from the final model.

Results

Reproductive Investment

We first investigated the effects of our treatments on two traits related to postcopulatory sexual selection: residual offspring survivorship and brood size (Table 3.1). A two-way ANOVA on residual offspring survivorship showed a significant effect of the mate size treatment, indicating that broods from large females were more successful than broods from small females, but we found no significant effect of food treatment on offspring survivorship (mate: $F_{1,30} = 5.13$, $P = 0.03$; food: $F_{1,30} = 0.0024$, $P = 0.96$; mate*food: $F_{1,30} = 0.43$, $P = 0.52$). Neither mate nor food treatment had a significant effect on brood size (two-way ANOVA, mate: $F_{1,32} = 0$, $P = 0.99$; food: $F_{1,32} = 0.33$, $P = 0.57$; mate*food: $F_{1,32} = 0.0013$, $P = 0.97$).

Table 3.1. **The effect of treatment on growth and offspring survivorship.** A summary of variables describing growth rate, post-copulatory sexual selection, and embryo development by treatment, showing mean (±S.E.) of each treatment goup.

Treatment	Percent Offspring Survivorship	Residual Offspring Survivorship	Brood Size	Pregnant Growth Rate, unstandardized (mm/day)	Pregnant Growth Rate, standardized (mm/day)	Mean Egg Size (mm^2)	Mean Embryo Length (mm)
Large Mate, High Food *n = 7*	79.72 (± 13.5)	1.87 (± 1.02)	35.29 (± 7.04)	0.222 (± 0.042)	0.005 (± 0.039)	0.914 (± 0.060)	6.69 (± 0.33)
Large Mate, Low Food *n = 8*	98.70 (± 0.66)	2.77 (± 0.78)	31.75 (± 8.92)	0.200 (± 0.028)	-0.081 (± 0.028)	0.972 (± 0.048)	6.9 (± 0.31)
Small Mate, High Food *n = 9*	88.66 (± 4.42)	-0.18 (± 1.53)	35.11 (± 20.76)	0.194 (± 0.047)	-0.013 (± 0.042)	0.876 (± 0.028)	5.9 (± 0.24)
Small Mate, Low Food *n = 10*	76.65 (± 9.88)	-0.95 (± 1.3)	32.00 (± 16.17)	0.133 (± 0.036)	-0.106 (± 0.041)	0.938 (± 0.032)	5.9 (± 0.30)
Overall *n = 34*	85.65 (± 4.26)	0.725 (± 0.66)	33.44 (± 2.76)	0.183 (± 0.02)	-0.053 (±0.02)	0.925 (±0.02)	6.3 (± 0.2)

A least squares linear regression (overall $R^2 = 0.32$, $F_{3,28} = 4.45$, $P = 0.01$) showed a significant effect of mate size ($b' = 0.35$, $P = 0.037$) and nearly significant

effects of brood size (Figure 3.1, *b'* = 0.32, *P* = 0.05) and latency to mate (*b'* = -0.30, *P* =0.07) on residual offspring survivorship. A previous study of Gulf pipefish also documented significant effects of these variables on offspring survivorship (Paczolt and Jones, 2010).

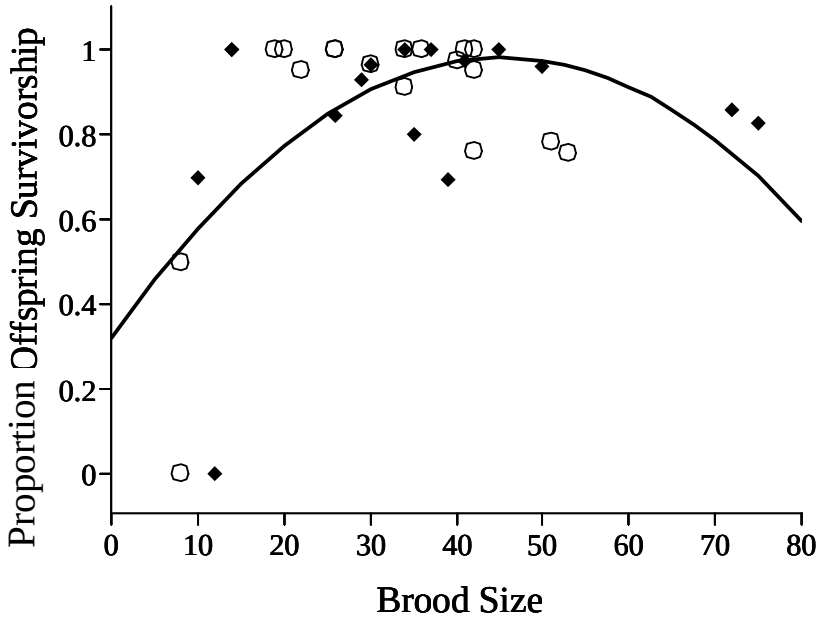

Figure 3.1. Broods of intermediate size are most successful, regardless of food treatment. Open circles are low food replicates, filled diamonds are high food replicates. AICc values were calculated following the method of Quinn and Keogh (2002), where the model with the smallest AICc value is the best fit of the data, and models with dAICc values greater than ten have no support. The curve shown is a quadratic regression of all replicates, which fits better than a linear regression (dAICc = 8.3) or a linear or quadratic regression of each treatment (linear regression, high food: dAICc = 61.0; linear regression, low food: dAICc = 53.8; quadratic regression, high food: dAICc = 59.6; quadratic regression, low food: dAICc = 34.8).

In short, we did find significant effects of female mate size on offspring survivorship during the male pregnancy in this experiment, but we did not find any

evidence that food treatment affected either brood size or offspring survivorship in Gulf pipefish. The lack of effect of food treatment is clear from a plot of raw offspring survivorship (i.e., number of surviving offspring divided by number of eggs initially received) against brood size (Figure 3.1). These relationships look virtually identical in the two food treatments (compare filled diamonds to open circles in Figure 3.1), and an analysis based on the Aikake information criterion (AIC) suggests that a quadratic regression model with observations lumped across all treatments provides the best fit to our data (Figure 3.1). Interestingly, this analysis suggests that an intermediate brood size provides higher offspring survivorship than either very small or very large broods, but our dataset contained only a handful of very large broods so additional work will be necessary to verify this pattern.

Growth Rate

Male growth rate before and during pregnancy was corrected for asymptotic growth patterns using the growth rates observed in control males (Table 3.1). Males grew less while pregnant than in the period before pregnancy (Figure 3.2, repeated measures ANOVA, within subjects, time: $F_{1,30} = 5.43$, $P = 0.017$), however there was no effect of mate or food treatment on this pattern (repeated measures ANOVA, within subjects, time*food: $F_{1,30} = 0.0002$, $P = 0.98$; time*mate: $F_{1,30} = 0.33$, $P = 0.57$).

We next investigated the effects of treatment on growth rate during pregnancy. Pregnant males in high food grew more than pregnant males in low food; however, mate treatment did not affect pregnant male growth rate (two-way ANOVA, mate: $F_{1,32} = 0.31$, $P = 0.58$; food: $F_{1,32} = 5.06$, $P = 0.03$; mate*food: $F_{1,32} = 0.008$, $P = 0.93$).

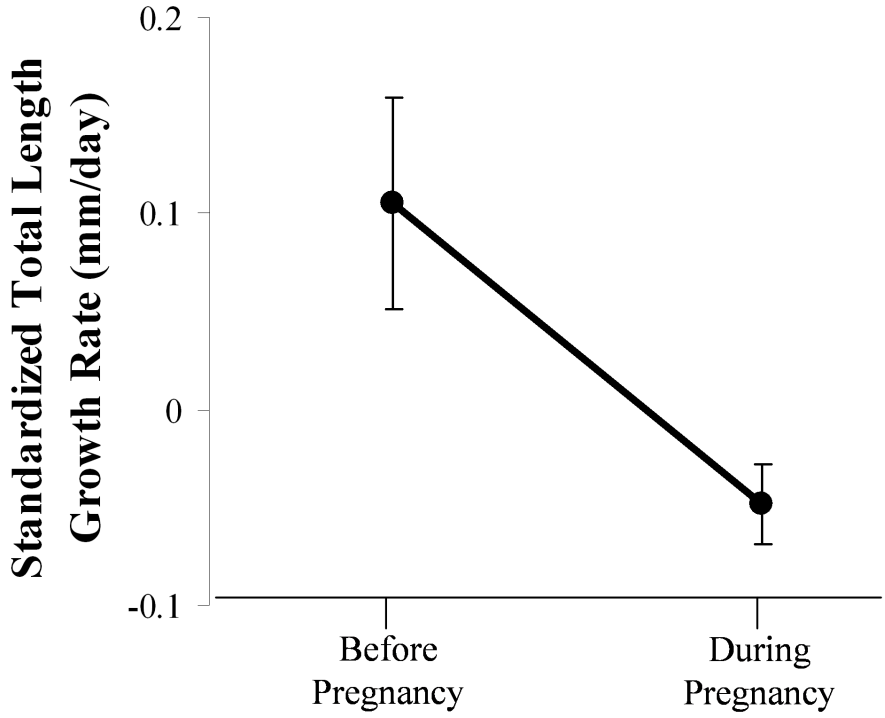

Figure 3.2. Males grow less during pregnancy than before. Males grow less during pregnancy than in the time period before (repeated measures ANOVA, within subjects, time: $F_{1,30} = 5.4317$, $P = 0.0167$).

We identified tradeoffs between growth and reproduction by using a least squares linear model of pregnant growth rate (overall model: $R^2 = 0.56$, $F_{7,26} = 4.76$, $P = 0.0015$). The direct effect of food treatment on pregnant male growth rate was highly significant ($b' = -0.39$, $P = 0.007$). The direct effects of non-pregnant growth rate ($b' = -0.078$, $P = 0.59$), latency to mate ($b' = -0.16$, $P = 0.30$), and number of failed eggs ($b' = 0.11$, $P = 0.46$) were nonsignificant. However the model showed significant interaction terms between food treatment and non-pregnant growth rate ($b' = 0.33$, $P = 0.028$), food treatment and latency to mate ($b' = -0.51$, $P = 0.002$), and food treatment and number of failed eggs ($b' = 0.59$, $P = 0.0006$).

To better understand the direction of these interaction effects, we ran a separate model of pregnant growth rate for each level of the food treatment. In high food (overall model: $R^2 = 0.34$, $F_{3,12} = 20.6$, $P = 0.16$), there are no significant effects of any of the variables (number of failed eggs: $b' = -0.52$, $P = 0.07$; non-pregnant growth rate: $b' = -0.37$, $P = 0.16$; latency to mate: $b' = 0.33$, $P = 0.21$). However, in low food (overall model: $R^2 = 0.63$, $F_{3,14} = 8.02$, $P = 0.002$), we found a significant effect of number of failed eggs ($b' = 0.75$, $P = 0.002$) and latency to mate ($b' = -0.79$, $P = 0.001$), as well as a marginally nonsignificant effect of non-pregnant growth rate ($b' = 0.31$, $P = 0.086$), showing that the significant effects in the overall model are driven by the low food treatment. The most interesting result from this analysis is that males in the low food treatment with a greater number of failed eggs in their brood pouches grew more than males with a smaller number of failed eggs, but this effect is not apparent in the high food treatment.

Egg and Embryo Size

We addressed the effect of resource availability and female size on the developing embryos by measuring embryo length and yolk area in photos of five randomly chosen embryos and their yolks from each brood on the day the male was killed, as well as photos of five eggs dissected from the relevant female's ovaries. Egg area and yolk area did not differ by mate treatment or food treatment (two-way ANOVA, average egg size, mate: $F_{1,32} = 0.79$, $P = 0.38$; food: $F_{1,32} = 2.14$, $P = 0.15$; mate*food: $F_{1,32} = 0.0026$, $P = 0.96$; two-way ANOVA, average yolk size, mate: $F_{1,32} = 0.24$, $P = 0.63$; food: $F_{1,32} = 0.91$, $P = 0.35$; mate*food: $F_{1,32} = 0.096$, $P = 0.76$). At day 8 of pregnancy, average embryo sizes for broods in the large mate treatment were significantly longer than those in the small mate treatment, but average embryo lengths

were not affected by food treatment (two-way ANOVA, mate: $F_{1,29} = 9.39$, $P = 0.005$; food: $F_{1,29} = 0.15$, $P = 0.70$; mate*food: $F_{1,29} = 0.095$, $P = 0.76$). A least squares linear model for average offspring length (overall model: $R^2 = 0.56$, $F_{3,27} = 11.5$, $P < 0.0001$) showed significant effects for female total length ($b' = 0.63$, $P = 0.0001$), number of failed eggs ($b' = -0.45$, $P = 0.0018$), and average egg size ($b' = -0.38$, $P = 0.012$).

Discussion

The results of our study shed new light on the dynamics of male pregnancy in pipefish when energetic resources may be limited. We expected males to grow less during pregnancy under low food compared to high food conditions, and we also expected pregnant males to compensate for reduced resources by reducing investment in their current brood. These predictions are only partially supported by our results. Our data do provide evidence of a life-history tradeoff between current reproduction and somatic growth in pregnant male Gulf pipefish. Males grow less during pregnancy than before it (Figure 3.2), supporting the hypothesis that pregnancy is energetically costly and that pregnant males support their broods using resources that might otherwise be invested in somatic growth. A similar pattern has been previously observed in a related pipefish, *S. typhle,* where brooding males grew less than non-brooding males (Svensson, 1988). However, to our surprise offspring survivorship appears to be unaffected by paternal resource availability (Figure 3.1), at least at the resource levels used in the present study. This result suggests that pregnant male pipefish sacrifice investment in somatic growth, and therefore future reproduction, in favor of current reproduction.

Our statistical analysis of pregnant male growth rate further illustrates the tradeoffs between growth and reproduction in pipefish. In the high food treatment, none

of the variables in our model exhibited a significant relationship with male growth rate, indicating that these males appear to have had sufficient resources to invest fully in both growth and reproduction. However, we saw a very different pattern in the low food treatment, where a number of different variables did predict pregnant male growth rate. Perhaps the most interesting result in the low food treatment concerned a tradeoff between growth and reproduction, in which males with more failed eggs in their pouch actually grew faster than males with fewer failed eggs. This result, while restricted to the low food treatment, is the sort of pattern we would expect if males either conserve parental effort by reducing energetic investment into their brood or reclaim resources from failed eggs and invest these resources into somatic growth. Sagebakken et al (2010) showed that radioactively-labeled amino acids from the eggs were deposited in the muscle tissue of the brooding father in a related pipefish species. These findings provide a possible mechanism to explain the patterns we have observed here, and, together with our results, suggest that males may be able to harvest resources from the brood and then reallocate these resources for somatic growth and maintenance. This pattern may be analogous to the filial cannibalism observed in many teleosts with parental care (reviewed in Manica 2002).

We were also interested in the effect of our treatments on embryo growth during the first half of the pregnancy, which is the time when the difference between successful and unsuccessful embryos becomes clear. Embryo size at day 8 was primarily driven by a positive effect of female size and negative effects of number of failed eggs and egg size. This effect of female size and number of failed eggs can be explained by increased paternal provisioning, increased egg quality, or both. The negative effect of egg size on embryo size may be caused by variation in egg size within females of a given size.

Alternatively, the negative effect of egg size in the model may mean that large egg size is not a good predictor of egg quality. Regardless, these results may partially explain the preference of males for females of larger size (Paczolt and Jones 2010), as the eggs from larger females tended to produce larger embryos. A similar advantage to mating with larger females has been suggested for *S. typhle*, another pipefish in which males prefer to mate with larger females (Berglund et al. 1986; Ahnesjö 1992).

Despite the significant role of male resource investment in the developing brood, there is no effect of male size or food treatment on embryo size. Embryos still carried a substantial yolk on the day they were measured, which may indicate that the female contribution through the yolk is still playing a significant role on embryonic growth at this stage of development. It is possible that there is a stronger male effect on offspring size during the second half of the pregnancy when the resources from the yolk are diminished, and this effect could be observed if juveniles were measured at birth. In the broad-nosed pipefish, broods with heavier eggs or more failed eggs produce heavier newborn offspring (Ahnesjo 1992, 1996). However, further work in the Gulf pipefish will be necessary to evaluate the effects of paternal resource allocation and egg failure on the growth of the surviving offspring at multiple time points, both during and after parturition.

In summary, we have documented a life history tradeoff between growth and reproduction in pregnant male Gulf pipefish. We have shown that males maintain investment in their current brood, despite the costs associated with pregnancy, and instead sacrifice somatic growth, and ultimately future reproduction, in resource-limited environments. Furthermore, males appear to be capable of recovering resources from failed eggs in their broods, and investing those resources in somatic growth. These

findings add to our understanding of the brood pouch as a key adaptation with far-reaching affects on the reproductive physiology and behavioral ecology of this interesting group of fishes.

CHAPTER IV

MALE PIPEFISH DIFFERENTIALLY ALLOCATE PARENTAL CARE BASED ON PERCEIVED FEMALE ATTRACTIVENESS

Introduction

Cryptic choice has been a controversial phenomenon since it first appeared in the scientific literature, in no small part because of how difficult it is to measure (Thornhill 1983, Birkhead 1998). Despite its elusive nature, opportunities abound for cryptic choice to occur through physiological or behavioral mechanisms that impact gamete usage, embryo survival, or the differential allocation of parental care (Eberhard 1999, Burley 1988, Sheldon 2000). Pitnick and Brown (2000) recommend manipulating female perception of male quality in order to study female effects, i.e. cryptic female choice, on reproductive success while controlling for male effects, i.e. sperm competition. In this study, we have adapted this approach in order to study whether perceived mate attractiveness can have a sustained effect on male investment into parental care in the male-pregnant Gulf pipefish.

Several studies have successfully manipulated perceived mate attractiveness and shown immediate effects on cryptic female choice. Experimental manipulation of paternal attractiveness, by decreasing UV intensity of the crown feathers, resulted in decreased maternal care in pair-bonded blue tits (Limbourg et al 2004). Similar mechanisms for fine-tuning cryptic choice have been found in a variety of bird species however this is possible only in species where the female regularly encounters her mate

after copulation. In another study, female guppies exerted cryptic choice in favor of male coloration by accepting more sperm from males they perceived to be more brightly colored (Pilastro et al 2004). In both of these cases, cryptic choice is modulated immediately after collecting mate choice cues; however, in many species mechanisms of cryptic female choice could continue to affect male reproductive success long after the mated pair parts ways. This seems particularly likely in species where the choosy parent continues investing in their offspring for a prolonged period of time, as in species with parental care.

Many of the pipefish species of the family Syngnathidae are sex-role reversed and have an elaborate form of male parental care, called male pregnancy. In sex-role reversed species, males are choosy and females compete for access to males. As a result, sexual selection acts more strongly on females. Pipefish females transfer unfertilized eggs to the male during copulation, after which he incubates the eggs in a specialized epithelial structure called the brood pouch. The male supports the developing brood by providing nutrition, protection, osmoregulation, and aeration (Azzarello 1991, Haresign and Schumway 1981, Partridge et al 2007, Quast and Howe 1980, Ripley 2009, Ripley and Foran 2009). This complex and energetically costly form of parental care provides an opportunity for cryptic male choice via differential allocation, a hypothesis supported by our previous results (Paczolt and Jones 2010).

In the sex-role reversed Gulf pipefish, *Syngnathus scovelli*, both pre- and postcopulatory phases of sexual selection favor large females. Males prefer to mate with large, active females (Jones and Avise 1997, Partridge et al, *in prep*) and offspring survivorship is biased in favor of these large females (Paczolt and Jones 2010). In this experiment, we exploit this preference for large females in order to manipulate male

perception of female quality. Males in our experiment encountered their mate in the presence of either a large stimulus female, causing the focal female to look less attractive, or a small stimulus female, causing the focal female to look more attractive. By comparing two males mated to the same female, we can examine the patterns of offspring survivorship in terms of the male's perception of female quality while controlling for variation in innate quality of either the females or the eggs. We expect that males mating in the presence of a large stimulus female will have reduced offspring survivorship while males mating with the same female in the presence of a small stimulus female will have increased offspring survivorship.

Methods

Sexually mature female and male Gulf pipefish were collected from Port Aransas, Texas in October 2010 and between July and October 2011 and transported back to our fish care facility at Texas A&M University. Fish were maintained in a saltwater recirculating system until all the males had given birth. The fish were then assigned to a replicate, and moved to individual 9.5 L home tanks equipped with sponge filters and artificial grass. Throughout the experiment, fish were fed two day old enriched Artemia nauplii twice daily, supplemented with copepods (OceanPods) once daily.

Each replicate was composed of two size matched males (80 - 100 mms, ±3 mm within pairs) one focal female (100 - 110 mm total length) and two stimulus females, one large (111 - 125 mm total length) and one small (85 - 99 mm total length). Each stimulus female was used in three trials. Each replicate included two mate choice trials, spaced four days apart. During each trial, one of the males was presented with the focal

female (of medium size) and one of the stimulus females (of either small or large size). At the end of the replicate the two males have mated with the same female in different social environments.

Mate choice trials were performed in 19 L tanks divided into one front and two rear compartments. The two rear compartments, which housed females, were separated using an opaque solid divider, visually isolating competing females from one another. The front compartment, which housed the male, was separated from both of the rear compartments with perforated, transparent, removable barriers. The rear and side walls of the tank were covered with white paper and the bottom of the tank was covered with a layer of crushed coral.

Fish were moved into the mate choice trial tanks during the evening before the trial, in order to acclimate the fish to the trial tank. Trials were run in the first two hours after lights on. Males spent the night in an opaque solid plastic cylinder with open ends that was placed at the center of the front compartment, thereby preventing the male from gathering most mate choice cues from the females before the beginning of the trial. The cylinder was lifted, releasing the male, five minutes before the mate choice trial began. Mate choice trials were recorder for one hour, after which the divider between the male and focal female was lifted. The three fish were left in the display tank for 24 hours to allow the male and focal female to mate. At the end of the 24 hours, all three fish were removed from the tank, measured, and returned to their home tanks. Stimulus females were allowed at least 24 hours to rest between trials, and focal females were allowed three days to rest between trials.

On pregnancy day 8, the male was killed with an overdose of MS-222 and the embryos were dissected from the pouch. At this stage of pregnancy, the difference between successful, normal embryos and unsuccessful, undeveloped eggs is unambiguous (Paczolt and Jones 2010). Percent offspring survivorship was calculated by dividing the number of successful eggs by the total number of eggs transferred and standardized using the standard arcsine square root transformation.

Male behaviors were scored from digital video using JWatcher version 1.0 animal behavior software (University of California Los Angeles, Los Angeles, CA: http://www.jwatcher.ucla.edu/). Twitching (abrupt shimmy of the whole body), dancing (rigid vertical swimming, often with a bobbing motion), and swimming near the divider were measured as in Partridge et al (2010). In addition, the amount of time swimming near the tank side wall and active time (any movement along the bottom of the tank) were also measured.

We performed 36 replicates in 2010 and 36 replicates in 2011. Of the 72 replicates total, 25 replicates had two successful matings and 15 had no successful matings in the time allotted. In 20 replicates, only the first male mated, and in 12 replicates only the second male mated. Neither order nor treatment significantly affected the probability of success for a mating (treatment: Fisher's exact test, 2-tail $P = 0.86$; order: Fisher's exact test, 2-tail $P = 0.23$). Our analysis will focus on the 25 replicates with two successful matings. Two of the twenty-five replicates with extremely large broods or high brood reduction were statistical outliers in the dataset and were excluded from the analyses.

Statistical analyses were performed in JMP, Version 9.0 (SAS Institute, Inc.). All behaviors were treated as the proportion of time spent doing a particular behavior towards the focal (or medium sized) female and standardized using the arcsine square root transformation. The advantage of this method is that it provides a measure of preference for the focal female (ranging from zero to one before transformation, one indicating a male that performed that behavior solely for the focal female) that is simultaneously standardized for variation between males in the amount of time doing any particular behavior. However, the drawback for this method is that males that did not perform a particular behavior could not be assigned a score for that behavior. Male behaviors were summarized using principal components analysis. The correlation and covariance matrix provided similar results for our data; the results from the correlation matrix are presented here. PCs 1 and 2, which cumulatively explained 93% of the variation in offspring survivorship (Table 4.1), were saved for further analyses. Missing values in the PCs were estimated using imputation, which calculates PC score using the partial information available.

Results

We first used repeated measures ANOVA to address whether order or treatment had an effect on brood size or offspring survivorship. Within replicates, second males typically had fewer eggs than first males (repeated measures ANOVA, within subjects, time: $F_{1,22} = 0.848$, $P = 0.0007$), however there was no effect of order on offspring survivorship (repeated measures ANOVA, within subjects, time: $F_{1,22} = 0.012$, $P = 0.637$). There was also no effect within or between replicates of stimulus treatment on either brood size (repeated measures ANOVA, between subjects, treatments: $F_{1,22} = 0.002$, $P = 0.85$; within subjects, time*treatment: $F_{1,22} = 0.002$, $P = 0.82$) or offspring

survivorship (repeated measures ANOVA, between subjects, treatment: $F_{1,22} = 0.0019$, $P = 0.85$; within subjects, time*treatment: $F_{1,22} = 0.0132$, $P = 0.622$).

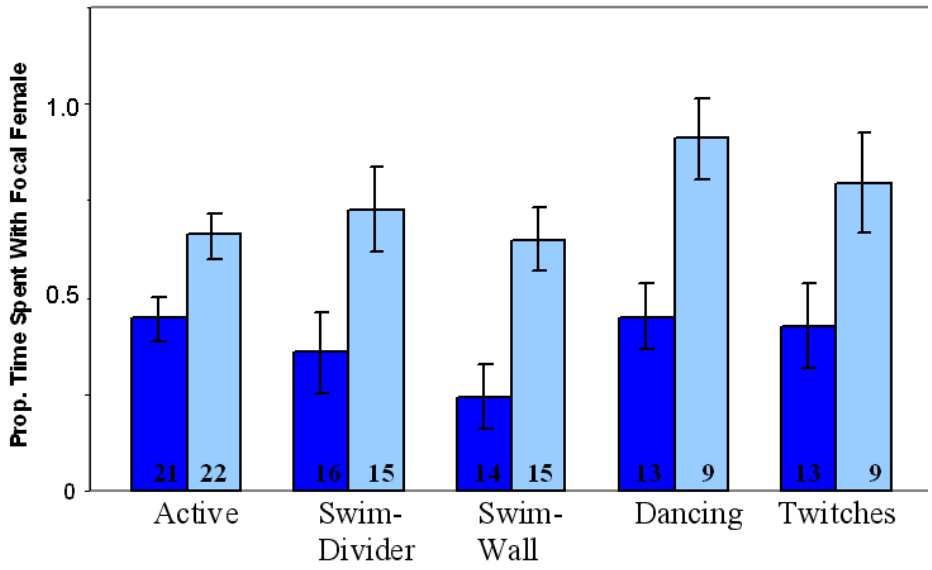

Figure 4.1. Males prefer the largest female available. Males in the large stimulus female treatment (dark blue bars) spend less time performing all recorded behaviors towards the focal female compared to males in the small stimulus female treatment (light blue bars). The sample size for each behavior is recorded within the bars; the maximum sample size is 23.

Males typically preferred their mate when in the presence of a small female rather than in the presence of a large female. Males spent more time performing all of the recorded behaviors toward the medium-sized focal female when a smaller stimulus female is present and less time when a larger stimulus female is present (Figure 4.1, Student's t-tests, active: n = 43, $P = 0.0156$; swim-wall: n = 31, $P = 0.0198$; swim-divider: n = 29, $P = 0.0015$; dancing: n = 21, $P = 0.0026$; twitches: n = 22, $P = 0.0433$). We next used principal components analysis to summarize male behaviors. All behaviors had high, positive loadings on PC1. PC2 is positively related to dancing and

twitching and negatively related to swimming near the divider or wall (Table 4.1). Males also scored higher on PC1 when in the presence of a small female than in the presence of a large female (Student's t-test, PC1 score, n = 46, $P = 0.0071$); however there was no effect of stimulus female size on PC2 (Student's t-test, PC2 score, n = 46, $P = 0.6771$).

Table 4.1. Variable loadings on the principle components analysis. This table summarizes the relationship between observed male behaviors and principle components analysis.

	Loading scores	
Behaviors	PC1	PC2
Active	0.940	-0.044
Swimming- Wall	0.878	-0.419
Swimming- Divider	0.863	-0.408
Dancing	0.879	0.423
Twitching	0.865	0.451
Percent Variance Explained	78.41%	14.54%
Eigenvalue	3.92	0.73
ChiSqaure	256.5	78.4
P value	<0.0001	<0.0001

In order to investigate the relationship between preference and offspring survivorship, we calculated the difference in male behavior and difference in offspring survivorship between males within a replicate. We found a significant positive relationship between PC2 and offspring survivorship (Figure 4.2, linear regression, $R^2 = 0.2806$, $F_{1,21} = 8.1929$, $P = 0.0093$). Within replicates, the male that had a stronger preference for the focal female also had higher offspring survivorship.

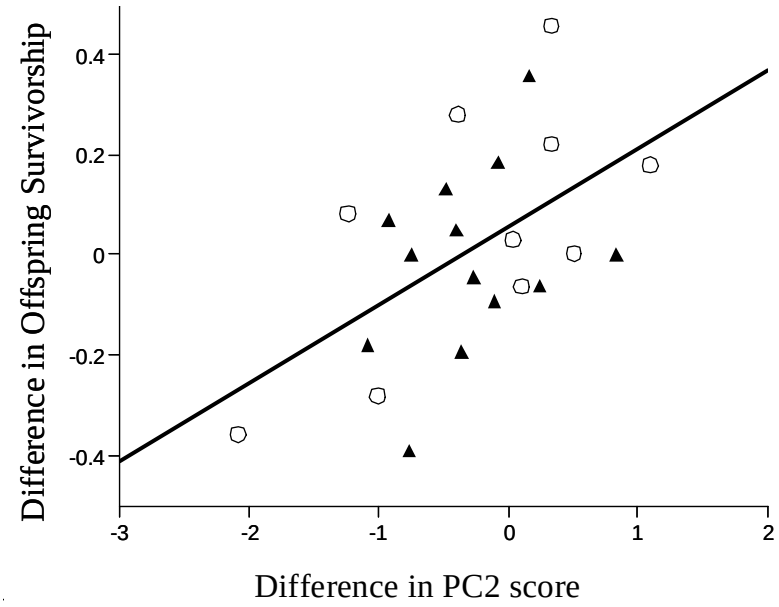

Figure 4.2. Mate attractiveness increases offspring survivorship. Within replicates, males that prefer their mate more also have higher offspring survivorship. The x-axis represents the difference in PC2 score between male 2 and male 1 of a replicate. The y-axis represents the difference in offspring survivorship within a replicate. Open circles represents replicates where the stimulus in the first trial was large and the stimulus female in the second trial was small. Filled squares represent replicates where the stimulus in the first trial was small and the stimulus female in the second trial was large.

Discussion

Our data supports the hypothesis that cryptic male choice acts in the Gulf pipefish via differential allocation of male parental care. Our previous study hypothesized that cryptic male choice is the mechanism driving patterns of postcopulatory sexual selection in the Gulf pipefish; however at that time we could not conclusively eliminate an alternative hypothesis citing female effects as the driving force behind the patterns we observed. By adapting the experimental design suggested by Pitnick and Brown (2000), we were able to compare the offspring survivorship of eggs from the same female carried in different males, thereby controlling for variation in female or egg quality. We also size matched males within each replicate in order to

control for variation between males mated to the same female, since our previous study showed that male total length is related to offspring survivorship (Paczolt and Jones 2010). Our analysis focuses on the remaining variation in offspring survivorship within replicates. The strong positive relationship between offspring survivorship and male courtship behavior, as shown in figure 4.2, supports the hypothesis that cryptic male choice is acting on offspring survivorship in this species. Furthermore, this result shows that the perception of mate attractiveness at the time of copulation can continue affecting mechanisms of cryptic male choice long after the mated pair has parted ways.

We found PC2 score to be a better predictor of offspring survivorship than the stimulus female size treatment we used. Although all assayed male behaviors were biased towards the largest female in the mate choice trial (Figure 4.1), this pattern did not translate to an effect of treatment on offspring survivorship. Previous studies have shown that males prefer the most active female available (Partridge et al *in prep*), which may explain the pattern we observed in our data. It is likely that in many cases the larger female was also more active; however we do not expect this to always be the case.

It is interesting to note that offspring survivorship is related to PC2 score rather than PC1 score, which explains a much greater proportion of the data (Table 4.1). This could be an effect of the high degree of intercorrelation among the assayed behaviors, as indicated by the high positive loadings for all behaviors on PC1. Essentially, PC1 can be interpreted as the total activity of the male on the side of the tank with the focal female. On the other hand, PC2 introduces contrast between behaviors. Time spent dancing or twitching, which are active courtship behaviors, load positively on PC2 while time spent swimming near the divider or wall of the tank load negatively on PC2. We can interpret the PC2 scores as distinguishing between the time spent actively courting and the time

spent swimming but not courting. From this perspective, our results indicate the offspring survivorship is best predicted by the tradeoff between courtship behaviors and non-courtship swimming behaviors (PC2), rather than overall male activity (PC1).

A similar effect of social environmental on parental care has been observed in a species with traditional sex roles where the competitive sex performs parental care. In bluegill sunfish, males in mating arenas with caged sneaker males performed less parental care than males with no sneaker males present (Neff 2003). In this case, males reduce parental care because of assumed cuckoldry rather than as a mechanism of cryptic choice on the female. Regardless, this study provides a complimentary example to our study because it shows how environmental cues at the time of copulation can impact parental care investment after those cues are no longer present.

We have shown here that the effects of mate preference can persist after copulation through mechanisms of cryptic choice in the Gulf pipefish. This case is useful to the study of postcopulatory sexual selection because examples of cryptic choice via differential allocation of parental care are comparatively rare in the scientific literature (Burley 1988, Sheldon 2000) despite the fact that we expect that opportunities for mechanisms of cryptic choice to evolve are common (Eberhard 1999). Furthermore, while our results address the effects of cryptic choice on the survivorship of embryos during the pregnancy, the effect of cryptic male choice may extend beyond offspring survivorship and manifest itself as paternal effects on offspring traits such as juvenile and adult growth rate or survival (Sheldon 2000). Additional studies would be necessary to explore the extent to which differential allocation impacts offspring fitness in this species. Finally, our results further explore the role of sexual selection and sexual

conflict within the brood pouch of syngnathid species, a trait that is proving to be more complex and intriguing with the analysis of each additional dataset.

CHAPTER V

DISCUSSION

In these studies we have investigated the role of postcopulatory sexual selection in the evolution of the male pregnant Gulf pipefish. We have shown that postcopulatory sexual selection does occur by differential allocation of paternal resources. These results are exciting because they expand our knowledge of the function and evolutionary history of this unique mode of reproduction and provide a new example of how postcopulatory sexual selection may manifest in a sex-role reversed species.

We first showed that males bias parental investment towards broods from large, attractive females, to the detriment of future broods. This result was the first to suggest that the pregnant male may mediate offspring survivorship through differential allocation of parental care resources and furthermore that sexual conflict may have a role in the evolution of male pregnancy.

Next, we investigated the effect of environmental factors on the patterns of offspring survivorship we have previously observed. We showed that in resource-poor environments, males will sacrifice investment in somatic growth in order to maintain investment in current reproduction and, in the process, indirectly sacrifice future reproduction. These results aid us in understanding how the male's reproductive tactic may change in the face of an ever-changing environment.

Finally, we confirmed that males exert cryptic choice according to female attractiveness, rather than inherent female quality, thereby conclusively eliminating the

hypothesis that offspring survivorship in the Gulf pipefish is driven by female manipulation of male parental care allocation. Furthermore, we saw that the effects of female attractiveness can have prolonged effects on the allocation of parental care in this species. In most examples of cryptic choice, mate attractiveness has a short-term effect on cryptic choice. Our data show that, at least in some species, this effect could be prolonged.

These results broaden our understanding of sexual selection and the evolution of male pregnancy in syngnathid fishes. Although we previously knew that the pregnant male invested resources in his brood, it was generally assumed that the male cared equally for all of his broods. These studies show that the male's preference for his mate continues to influence the female's reproductive success after copulation, extending to the dynamics occurring within the pregnancy itself. Furthermore these dynamics are labile and responsive to changes in the male's environment. Our research is the beginning of a new and exciting area of study for syngnathid biologists because these studies raise many new questions about the ecology and evolution of these unique fishes. Furthermore, these studies add a crucial example of sex-role reversed cryptic choice to the literature on postcopulatory sexual selection and supports the idea that with closer examination we may find that postcopulatory sexual selection is a pervasive phenomenon affecting many species.

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VITA

Name:	Kimberly Ann Paczolt
Address:	1210 Biology-Psychology Building, Department of Biology, University of Maryland, College Park, Maryland, 21742
Email Address:	kpaczolt@umd.edu
Education:	B.S., University of Illinois at Urbana-Champaign, 2004